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(54) Title: AGENT FOR REDUCTION OF SCAR FORMATION BY USING WOUND ALKALINIZATION

(57) Abstract: The present invention relates to an agent for reducing scar formation, which inhibits the scar formation by injecting sodium bicarbonate to the dermis tissue of the wound with a syringe to directly control pH of the wound site, namely, by alkalinizing the wound to inactivate TGF- β . According to the present invention, the agent for reducing scar formation by controlling the wound healing process can be clinically and immediately applied to the treatment of tylosis scar and keloid, etc., and is effective to the treatment of intractable fibrosis diseases. Also, the invention can be widely applied in many ways as an easy tool which can control the action of TGF- β in a living organism.

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**AGENT FOR REDUCTION OF SCAR FORMATION
BY USING WOUND ALKALINIZATION**

TECHNICAL FIELD

5 The present invention relates to an agent for reducing scar formation by using alkalization of the wound, which is clinically easy to apply. More particularly, the present invention relates to an agent for reducing scar formation, which inhibits the scar formation by injecting sodium bicarbonate to the dermis tissue of the wound with a syringe to directly control pH of the wound site, namely, by alkalinizing the wound to inactivate TGF- β .

10 **BACKGROUND ART**

There have been efforts to reduce the formation of scar. However, an effective method has not yet been found. It was reported that the wound of a fetus is cured without scar. After such report, trials have been made to reveal the differences between the wound healing process in a fetus and that in a mature individual, change the wound healing process in a mature individual to that in a fetus, and thereby reduce the scar formation. One of the revealed differences is that Transforming Growth Factor-Beta (TGF- β) is not immunochemically detected in the wound of a fetus, but functions in the wound healing process of a mature individual and plays an important role in the scar formation. This fact shows that it would be possible to inhibit the scar formation by controlling TGF- β . Actually, there was a report that the scar formation in a white rat could be reduced by the use of an antibody against TGF- β (*Acidic cellular environments; activation of latent TGF-beta and sensitization of cellular responses to TGF-beta and EGF. International Journal of Cancer. 43(5):886-91, 1989 May 15*). TGF- β is secreted in inactivated state from cells and is mostly present in inactivated state even outside of cells. Only when exposed to a certain specific condition, TGF- β is activated, combines with a receptor of a target cell and functions. Heat, acidic condition, protease, etc. are known as physicochemical conditions which can activate TGF- β (*Physicochemical activation of recombinant latent transforming growth factor-beta's 1, 2, and 3. Growth Factors. 3(1):35-43, 1990*). However, no studies have been made yet

with respect to the reduction of scar formation by using the inactivation of TGF- β . The present invention aims to control the pH of the wound, which is clinically easy to apply, namely, to alkalinize the wound and lead the inactivation of TGF- β , and to confirm whether the inactivation actually reduces the scar formation.

5 There have been several trials to reduce scar formation by controlling TGF- β which functions during the entire process of scar formation. However, those trials were related to the use of an antibody and have problems in actual application. TGF- β widely functions in a living organism and is under active study in various fields. The processes of formation, secretion and function of TGF- β have not yet been clearly known. According to the facts
10 discovered to date, TGF- β is secreted from cells in inactivated state and functions with a target organ under specific conditions. Heat, acidic pH, and protease are known as the physicochemical conditions which can activate TGF- β . However, no trials have been made to reduce the scar using TGF- β .

DISCLOSURE OF INVENTION

15 The present invention was conceived based upon the above stated facts. The inventors of the present invention have completed the present invention by controlling the pH of the wound, which is clinically easy to apply, that is, by alkalinizing the wound and inactivating TGF- β , thereby leading the wound healing process which is similar to that of a fetus and confirming that the scar formation is reduced. Accordingly, the objective of the
20 present invention is to provide an agent for reducing scar formation, which inactivates TGF- β , reduces the scar formation and thus heals the wound without scar.

 The present invention is directed to a method for reducing scar formation during the wound healing process. TGF- β is known to play an important role in the scar formation process of the wound. To inactivate TGF- β , the wound should be alkalinized. To easily
25 alkalinize the wound, sodium bicarbonate in the concentration of sodium bicarbonate 1mEq (1cc)/distilled water 10cc is injected to the dermis tissue of the wound to directly control pH of the wound, thereby reducing the scar formation of the wound. The pH range which effectively reduces the scar formation, namely, which does not activate TGF- β and alkalinizes

the wound, is 9.0-10.0, preferably, 9.2-9.8. Also, sodium bicarbonate of the present invention reduces fibrosis in the process of tissue regeneration or healing, by controlling pH (alkalinization) of the regeneration or healing spot.

BRIEF DESCRIPTION OF THE DRAWINGS

5 Fig. 1a shows the tissue of the control group after two weeks from the injection of distilled water, instead of sodium bicarbonate(dyeing: hematoxylin-erosin dyeing, magnification: 40X).

 Fig. 1b shows the tissue of the test group after two weeks from the injection of sodium bicarbonate(dyeing: hematoxylin-erosin dyeing, magnification: 40X).

10 Fig. 2a shows the tissue of the control group after two weeks from the injection of distilled water, instead of sodium bicarbonate(dyeing: trichrome dyeing, magnification: 400X).

 Fig. 2b shows the tissue of the test group after two weeks from the injection of sodium bicarbonate(dyeing: trichrome dyeing, magnification: 400X).

 Fig. 3a shows the tissue of the control group after eight weeks from the injection of
15 distilled water, instead of sodium bicarbonate(dyeing: hematoxylin-erosin dyeing, magnification: 40X).

 Fig. 3b shows the tissue of the test group after eight weeks from the injection of sodium bicarbonate(dyeing: hematoxylin-erosin dyeing, magnification: 40X).

 Fig. 4a shows the tissue of the control group after eight weeks from the injection of
20 distilled water, instead of sodium bicarbonate(dyeing: trichrome dyeing, magnification: 400X).

 Fig. 4b shows the tissue of the test group after eight weeks from the injection of sodium bicarbonate(dyeing: trichrome dyeing, magnification: 400X).

 Fig. 5a shows the tissue of the test group after two weeks from the injection of TGF- β (dyeing: hematoxylin-erosin dyeing, magnification: 40X).

25 Fig. 5b shows the tissue of the test group after two weeks from the injection of both TGF- β and sodium bicarbonate(dyeing: hematoxylin-erosin dyeing, magnification: 40X).

 Fig. 6a shows the tissue of the test group after eight weeks from the injection of TGF- β (dyeing: hematoxylin-erosin dyeing, magnification: 100X).

Fig. 6b shows the tissue of the test group after eight weeks from the injection of both TGF- β and sodium bicarbonate(dyeing: hematoxylin-erosin dyeing, magnification: 100X).

Fig. 7a shows the tissue of the test group after eight weeks from the injection of TGF- β (dyeing: trichrome dyeing, magnification: 400X).

5 Fig. 7b shows the tissue of the test group after eight weeks from the injection of both TGF- β and sodium bicarbonate(dyeing: trichrome dyeing, magnification: 400X).

EXAMPLES

The following examples disclose the present invention in more detail.

10 In the wound healing process of an adult white rat, it was histologically studied whether the alkalization of the wound reduces the scar formation, and it was indirectly confirmed whether such a reduction is caused by TGF- β inactivation, by observing differences of scar leading action of TGF- β between the alkalized wound and the control wound.

MATERIALS

15 Animal: forty white rats of Sprague-Dawley origin, which weigh 300-350mg
bovine TGF- β
0.1 mEq sodium bicarbonate

METHODS OF EXPERIMENT

A. Experiment of wound alkalization (twenty white rats)

20 On the dorsum of adult white rats, skin near the limbs which are on the same distance from the median line was incised in about 10mm long. After incision, the control group was simply sutured. In sham control group, 0.1cc distilled water was injected to the dermis of the incised wound. In test group, 0.1 mEq sodium bicarbonate 0.1cc (Ph 9.5 ± 0.3) was injected to the dermis of the incised wound.

25 a. control x 1 site: suture only
b. experimental x 1 sites: 0.1 mEq Sodium bicarbonate0.1cc injection and suture

B. Experiment of TGF- β Inactivation (twenty white rats)

On the dorsum of adult white rats, skin near the limbs which are on the same distance from the median line was incised in about 10mm long. Before suture, TGF- β 0.05 μ g was injected to the dermis of the incised wound in test group I, while both TGF- β 0.05 μ g and 0.1 mEq sodium bicarbonate 0.1cc (Ph 9.5 \pm 0.3) were injected to the dermis of the incised wound
5 in test group II.

- a. experimental I x 1 site: TGF- β injection and suture
- b. experimental II x 1 sites: TGF- β + sodium bicarbonate
injection and suture

ANALYSIS OF THE RESULTS

10 A. Histological observation

On 14th and 56th days from the experiments, tissues of ten adult white rats were taken, fixed in 10% neutral buffer formalin for 24 hours, embedded in paraffin throughout the normal tissue treating process. In the middle of the wound, the tissue was cut into 4 μ m thick, perpendicularly to the major axis, and dyed with hematoxyline-eosine and Masson's trichrome
15 to observe the connective tissues (Figs. 1a to 7b).

In Figs. 1a and 1b, control group (a) shows a thicker scar, which is marked with an arrow, than the test group (b).

In Figs. 2a and 2b, control group (a) shows thin, compact, irregular blue-died collagen fibers, which are typical collagen appearance of scar. On the other hand, test group
20 (b) shows relatively thick, regular collagen fibers, which are relatively spacious compared to the control group (a).

In Figs. 3a and 3b, control group (a) shows a thicker scar, which is marked with an arrow, than the test group (b).

In Figs. 4a and 4b, control group (a) shows thin, irregular blue-died collagen fibers,
25 which are typical collagen appearance of scar. On the other hand, test group (b) shows relatively thick, regular collagen fibers, of which structures are almost similar to the normal dermis nearby.

In Figs. 5a and 5b, TGF- β injected group (a) shows a thicker scar, which is marked

with an arrow, than the test group (b).

In Figs. 6a and 6b, TGF- β injected group (a) shows a thicker scar, which is marked with an arrow, than TGF- β + sodium bicarbonate injected group (b). The scar of TGF- β + sodium bicarbonate injected group (b) is too thin to find, and the structure of the tissue is similar to the normal dermis nearby.

In Fig. 7a and 7b, TGF- β injected group (a) shows thin, irregular blue-died collagen fibers, which are typical collagen appearance of scar, and forms a thick scar. On the other hand, TGF- β + sodium bicarbonate injected group (b) shows very thin scar and its collagen fibers are thick, regular and of which structure is almost similar to the collagen structure of normal dermis nearby.

B. MEASUREMENT OF THE WIDTH OF SCAR

With a tissue sample, which was cut perpendicularly to the middle of the wound, the width of the scar by use of image analysis program (Image-Pro version 3.0, Microsoft) under 40 magnification was measured, and the differences between the groups were compared. The result of the measurement was confirmed by Student t-test.

RESULTS

The tissues to be tested were obtained from tested forty white rats, twenty of which were sacrificed at each time, after 2 and 8 weeks from the operational handling.

A. Experiment of wound alkalinization

After two (2) weeks from a wound was made, an inflammation was observed from the epidermal tissue layer to the muscular tissue layer at the low magnification (x 40) in hematoxylin-eosin dyeing, and there was not much difference between the degree of deposition of inflammatory cells of the test group and the control group. But the width of the scar of test group was thinner than that of the control group (Refer to Figure 1). The deposited Collagen of Trichrome dyeing was thin and irregular in both the test group and the control group. But the amount of deposition of the Collagen of the test group was meager compared to that of the control group, and deposition of the Collagen of the control group was larger and denser (Refer to Figure 2).

When the width of scar was measured at magnification of 40, that of the control group was average of $68.44 \mu\text{m} \pm 22.84$, and that of the test group was average of $26.66 \mu\text{m} \pm 9.60$. As a result of student t-test, the value of p at a significant level of 0.05 of the two groups was the value of 0.01 or less, which was statistically significant difference (Refer to Table 1).

In the hematoxylin-eosin dyeing of the wound after eight (8) weeks from the wound was made, the test group formed a thin and unnoticeable scar and the control group formed a thick and significant scar at the low magnification (x 40) (Refer to Figure 3). At the high magnification (x 400), the test group showed thicker and regular Collagen fibers which was not much different from the normal dermis nearby, whereas the control group showed compactly deposited Collagen fiber, which was clearly distinguishable from the normal dermis nearby. Not many of the fibroblast cells were found in the test group but many of them were still found in the control group. In Trichrome dyeing, the density of Collagen fiber in the test group was not different from that of the normal dermis nearby. The fiber was thick and regular, which was recovered closed to the dermis, while since thin and irregular fiber was densely deposited, the control group clearly showed the scar (Refer to Figure 4). When the width of the scar was measured at 40 magnification, that of the control group was average of $37.67 \mu\text{m} \pm 7.98$, and that of the test group was average of $13.89 \mu\text{m} \pm 2.42$. As a result of student t-test, the value of p at a significant level of 0.05 of the two groups was the value of 0.01 or less, which was a statistically significant difference (Refer to Table 1).

<Table 1>

Comparison of the width of sodium bicarbonate group and that of control group (measured by using image analysis program, "Image-Pro version 3.0, Microsoft")

	Control Group* (n=10)	sodium bicarbonate group* (n=10)	Value of p
2 Weeks	$68.44 \mu\text{m} \pm 22.84$	$26.66 \mu\text{m} \pm 9.60$	$0.01 \geq$
8 Weeks	$37.67 \mu\text{m} \pm 7.98$	$13.89 \mu\text{m} \pm 2.42.$	$0.01 \geq$

*Mean \pm SD

B. Experiment of TGF- β inactivation

After two (2) weeks from a wound was made, an inflammation was observed from epidermal tissue layer to the muscular tissue layer at the low magnification (x 40) in hematoxylin-eosin dyeing, and there was not much difference between the degree of deposition of inflammatory cells of TGF- β group and sodium bicarbonate + TGF- β group. But the width of the scar of sodium bicarbonate + TGF- β group was thinner than that of the TGF- β group (Refer to Figure 5). The deposited Collagen of Trichrome dyeing was thin and irregular in both the test group and the control group. But the amount of deposition of the Collagen of the test group was meager compared to that of the control group, and deposition of the Collagen of the control group was larger and denser.

When the width of scar is measured at magnification of 40, that of the TGF- β group is average of $74.84 \mu\text{m} \pm 15.93$, and that of sodium bicarbonate + TGF- β group was average of $35.41 \mu\text{m} \pm 6.90$. As a result of student t-test, the value of p at a significant level of 0.05 of the two groups was the value of 0.01 or less, which was a statistically significant difference (Refer to Table 2).

In the hematoxylin-eosin dyeing of the wound after eight (8) weeks from the wound was made, sodium bicarbonate + TGF- β group formed a thinner and less noticeable scar than TGF- β group at the low magnification (x 40). The control group formed a thicker and more significant scar than the test group at the low magnification (x 40) (Refer to Figure 6). At the high magnification (x 400) as same as the test of alkalinization of a wound, sodium bicarbonate + TGF- β group showed thicker and regular Collagen fibers which was not much different from the normal dermis nearby.

In Trichrome dyeing, the density of Collagen fiber in sodium bicarbonate + TGF- β group was not that different from the normal dermis tissue nearby. The fiber was thick and regular, which was recovered closed to the dermis tissue, while since thin and irregular fiber was densely deposited, TGF- β group clearly showed the scar (Refer to Figure 7). When the width of the scar was measured at 40 magnification, that of TGF- β group was average of $72.92 \mu\text{m} \pm 16.04$, and that of sodium bicarbonate + TGF- β group was average of $49.09 \mu\text{m} \pm$

5.58. As a result of student t-test, the value of p at a significant level of 0.05 of the two groups was the value of 0.01 or less, which was a statistically significant difference (Refer to Table 2).

<Table 2>

5 Comparison of the width of sodium bicarbonate group and that of the control group (measured by using image analysis program, "Image-Pro version 3.0, Microsoft")

	TGF- β *(n=10)	sodium bicarbonate + TGF- β * (n=10)	Value of p
2 Weeks	74.84 $\mu\text{m} \pm 15.93$	35.41 $\mu\text{m} \pm 6.90$	0.01 \geq
8 Weeks	72.92 $\mu\text{m} \pm 16.04$	49.09 $\mu\text{m} \pm 5.58$	0.01 \geq

*Mean \pm SD

10 INDUSTRIAL APPLICABILITY

As explained and shown in the Examples above, the agent for reducing scar formation according to the present invention by controlling the wound healing process can be clinically and immediately applied to the treatment of tylosis scar and keloid, etc., and is effective to the treatment of intractable fibrosis diseases. Also, the invention can be widely applied in many ways as an easy tool which can control the action of TGF- β in a living organism.

CLAIMS

1. An agent for reducing scar formation on wound comprising sodium bicarbonate, characterized by injecting sodium bicarbonate to the dermis tissue of the wound to directly control pH of the wound and heal the wound without scar.
- 5 2. The agent for reducing scar formation according to claim 1, wherein the reduction of scar formation is achieved by controlling pH of the wound to inactivate TGF- β .
3. The agent for reducing scar formation according to claim 1, wherein the pH of the wound is controlled to range from 9.0 to 10.0.
4. The agent for reducing scar formation according to claim 3, wherein the pH of the
10 wound is controlled to range from 9.2 to 9.8.
5. The agent for reducing scar formation according to any one of claims 1 to 4, wherein sodium bicarbonate is used in concentration of 1mEq (1cc)/distilled water 10cc.

FIG. 1a

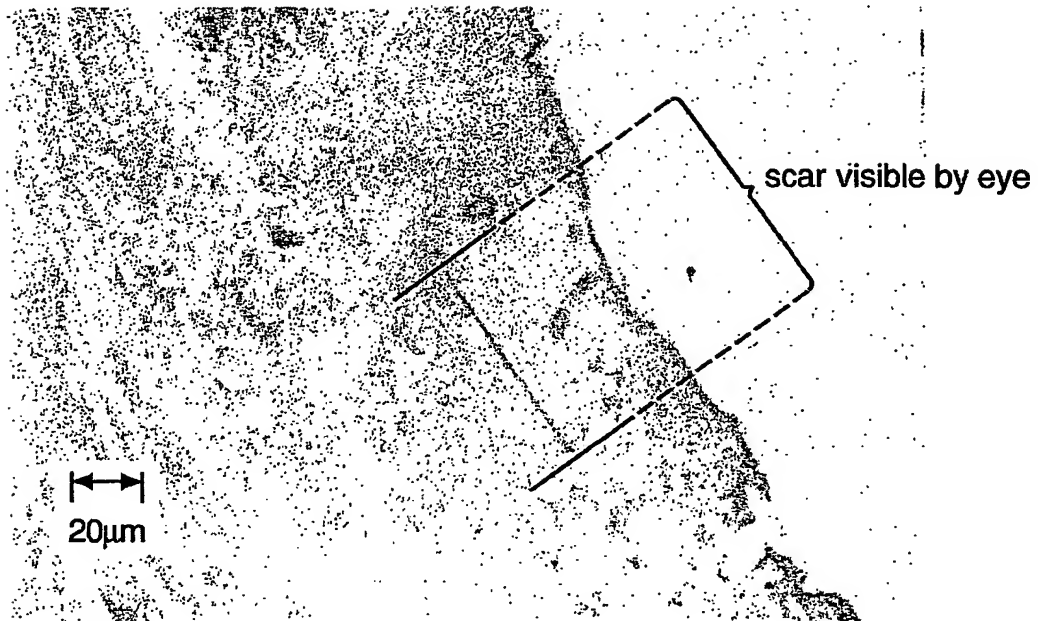
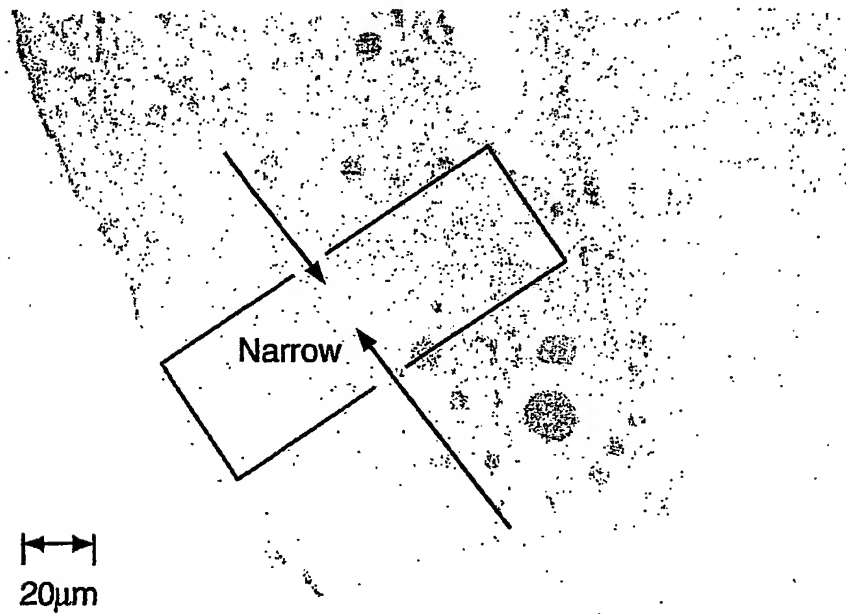


FIG. 1b



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FIG. 2a



FIG. 2b

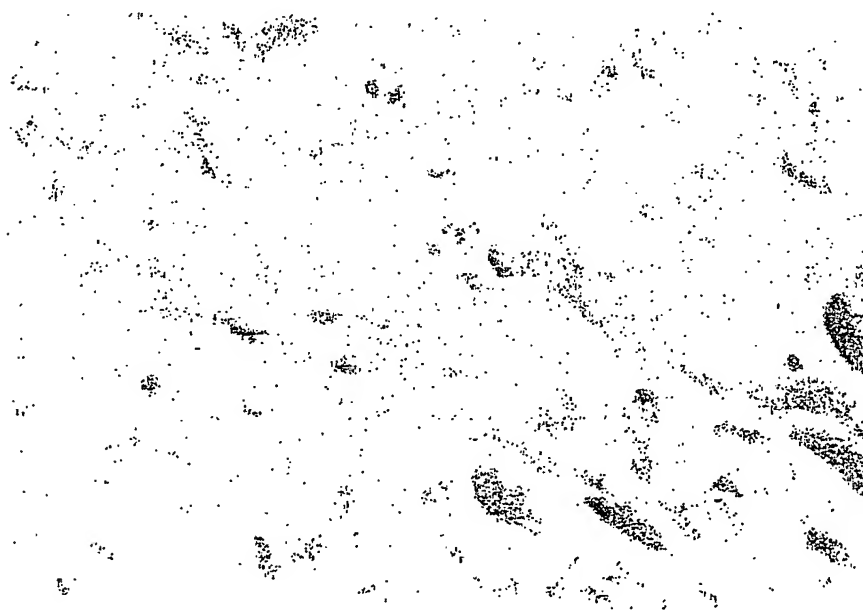


FIG. 3a

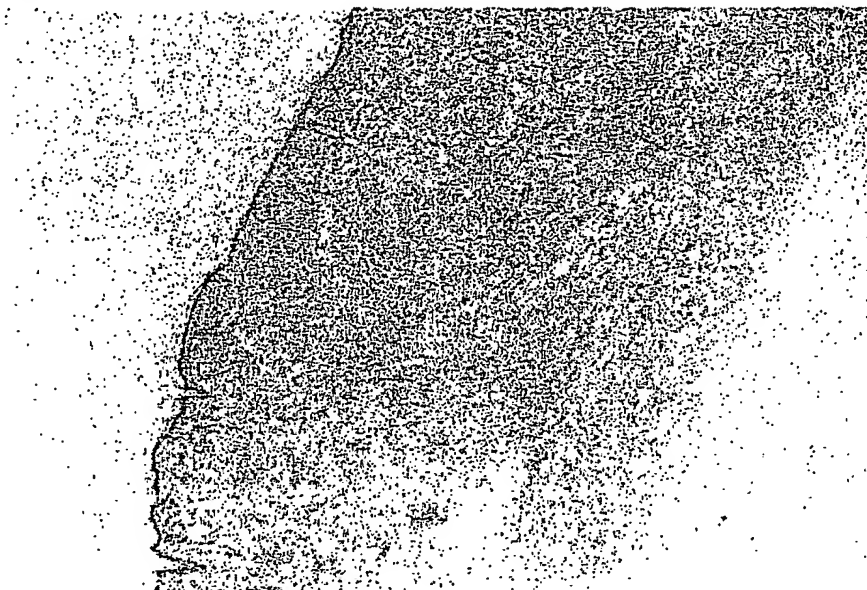


FIG. 3b

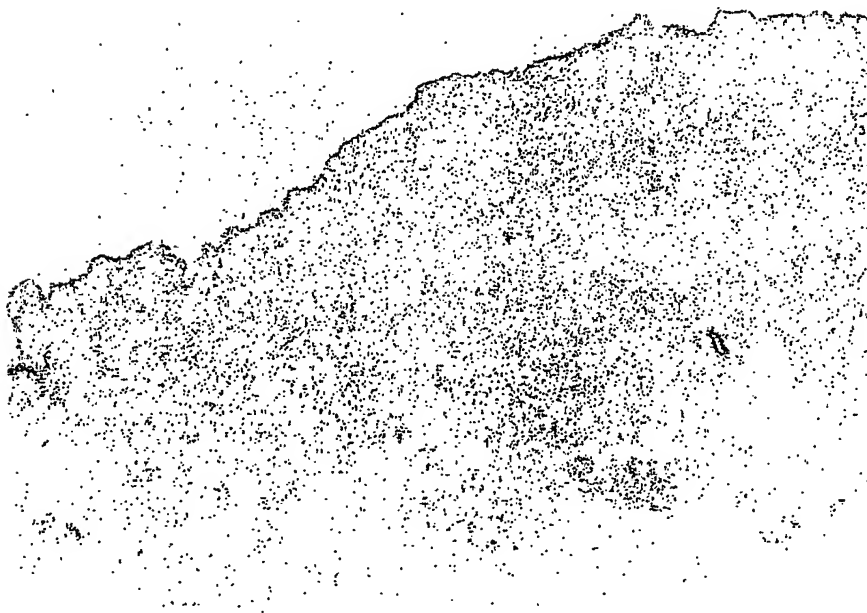


FIG. 4a

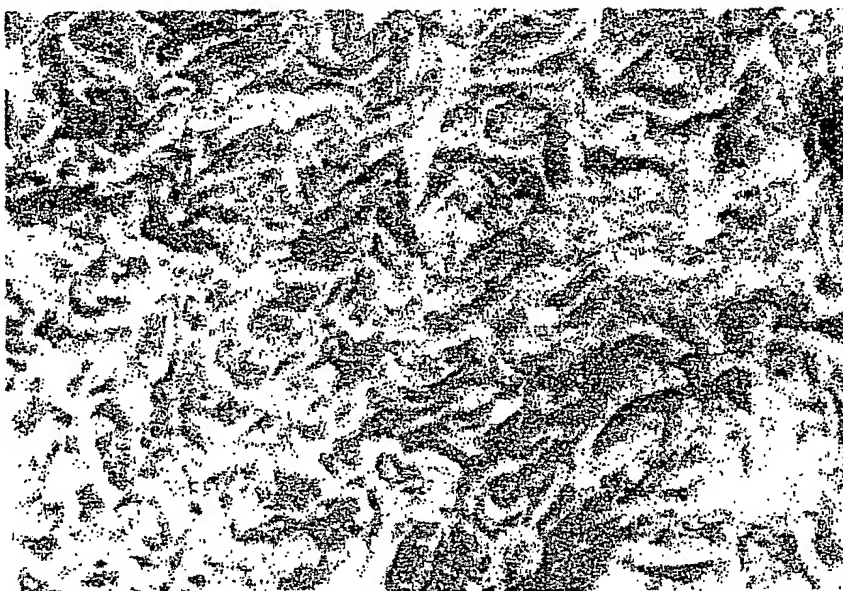
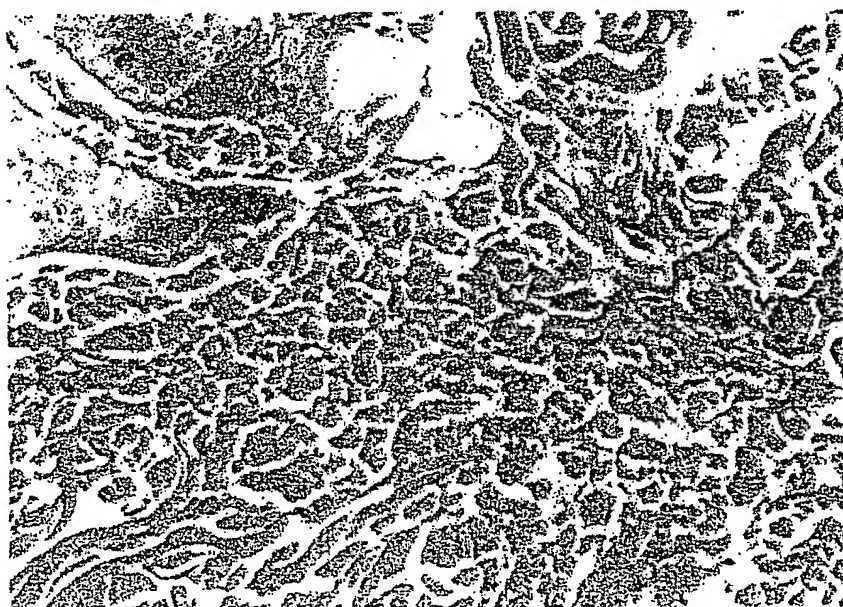


FIG. 4b



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FIG. 5a



FIG. 5b



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FIG. 6a



FIG. 6b

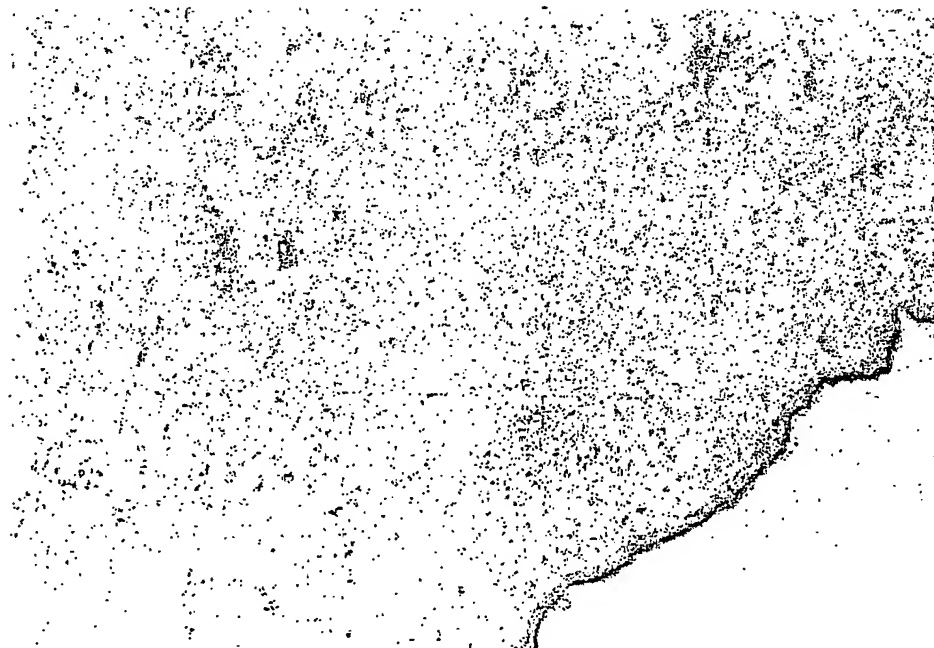


FIG. 7a

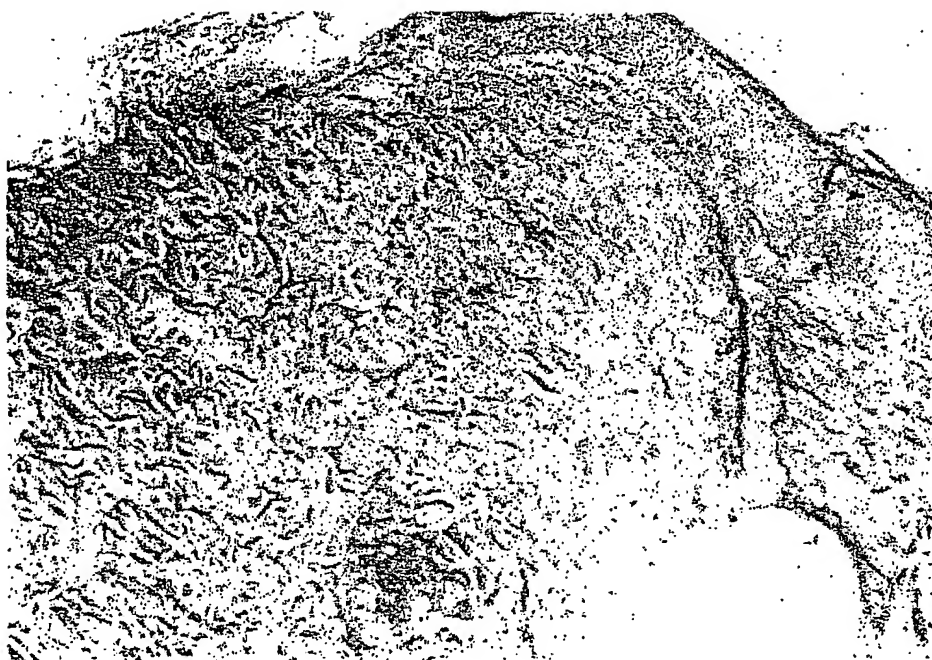
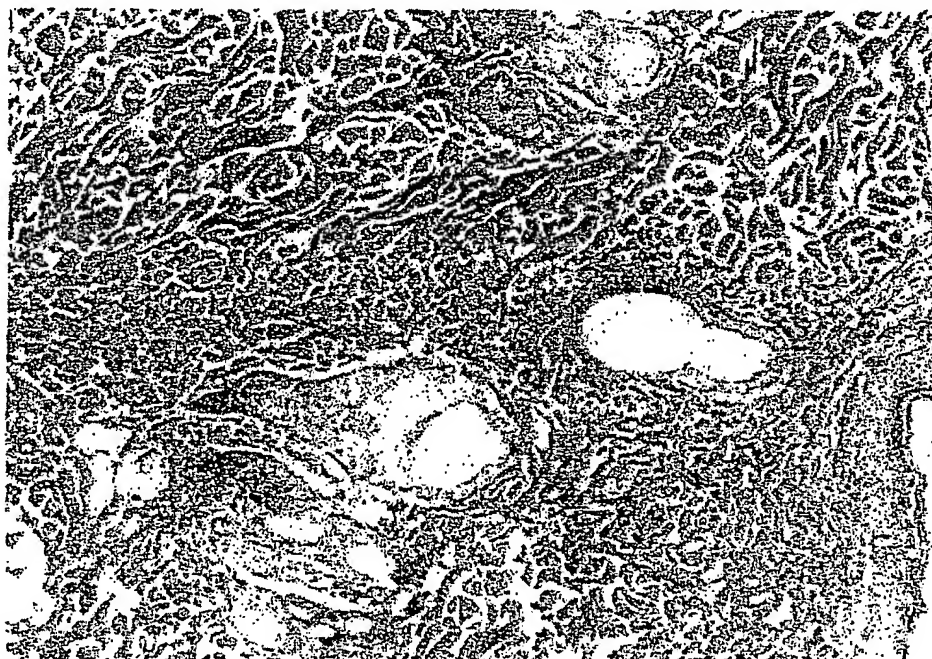


FIG. 7b



INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB01/01803

A. CLASSIFICATION OF SUBJECT MATTER														
Int. Cl. ⁷ : A61K 33/00, A61P 17/02														
According to International Patent Classification (IPC) or to both national classification and IPC														
B. FIELDS SEARCHED														
Minimum documentation searched (classification system followed by classification symbols)														
IPC: A61K AND KEYWORDS AS INDICATED BELOW														
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched														
AU: IPC AS ABOVE														
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)														
Derwent, Medline, Chemical Abstracts: sodium bicarbonate, nahco3, baking soda, bicarbonate of soda, scar+, keloid, dermis, inject+, parenteral, transforming growth factor-beta														
C. DOCUMENTS CONSIDERED TO BE RELEVANT														
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.												
X	Dermatosen In Beruf Und Umwelt (Occupational and Environmental Dermatoses), Vol. 32, No. 4, 1984, Fuchs et al, Schmutztatowierung nach Explosion im Chemieunterricht (Dirt Tattooing following an explosion in a Chemistry Class), pages 130-140 See especially abstract	1-5												
X	WO 93/08811 A (ENDERLE) 13 May 1993 See Especially claim 5	1-5												
X	FR 2770779 A (BERNSTEIN) 14 May 1999 Whole Document	1-5												
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C <input checked="" type="checkbox"/> See patent family annex														
<table border="0"> <tr> <td>* Special categories of cited documents:</td> <td>"I" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td> </tr> <tr> <td>"A" document defining the general state of the art which is not considered to be of particular relevance</td> <td>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td> </tr> <tr> <td>"E" earlier application or patent but published on or after the international filing date</td> <td>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td> </tr> <tr> <td>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td> <td>"&" document member of the same patent family</td> </tr> <tr> <td>"O" document referring to an oral disclosure, use, exhibition or other means</td> <td></td> </tr> <tr> <td>"P" document published prior to the international filing date but later than the priority date claimed</td> <td></td> </tr> </table>			* Special categories of cited documents:	"I" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	"E" earlier application or patent but published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family	"O" document referring to an oral disclosure, use, exhibition or other means		"P" document published prior to the international filing date but later than the priority date claimed	
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Date of the actual completion of the international search		Date of mailing of the international search report												
21 February 2002		27 FEB 2002												
Name and mailing address of the ISA/AU		Authorized officer												
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustalia.gov.au Facsimile No. (02) 6285 3929		R.L. POOLEY Telephone No : (02) 6283 2242												

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB01/01803

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	US 6063406 A (HORNACK) 16 May 2000 See especially Column 4	1
A	International Journal of Cancer, Vol. 43, No. 5, May 1989, P. Jullien et al, "Acidic Cellular Environments: Activation of Latent TGF- β and Sensitization of Cellular Responses To TGF- β and EGF", pages 886-891	
A	American Journal of Kidney Diseases, Vol 34, No. 2, August 1999, EA Baroni et al, "Sodium Bicarbonate Treatment Reduces Renal Injury, Renal Production of Transforming Growth Factor- β , and Urinary Transforming Growth Factor- β Excretion in Rats with Doxorubicin-Induced Nephropathy", pages 328-337	

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/IB01/01803

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member			
WO	93/08811	DE	19930506		
FR	2770779	NIL			
EP	497724	AT	135915	DE	69209335
		GR	3020175	US	5441748
				ES	2084971
				JP	5058900
CN	1156042	NIL			
CN	1270812	NIL			
US	6063406	NIL			
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